综 述

细胞外囊泡的分析方法及临床应用进展

安泰学,郑 磊
南方医科大学南方医院检验医学科,广东 广州 510515

摘要:细胞外囊泡(EVs)是近年来新兴的研究领域,是细胞释放的微小囊泡,其中含蛋白质、miRNA等生物学活性分子。研究表明,在多种疾病中体液EVs发生相应改变,能够灵敏反映体内病理变化情况。相较于组织活检,体液EVs检测具有无创、取样简便、可实时监测等明显优势,因而受到研究者以及临床医生的密切关注,有望成为新一代诊断标志物。本文将对EVs在临床应用方面的研究现状和进展作一简述。

关键词:细胞外囊泡;分离纯化;临床诊断;临床治疗;临床应用

Progress and analysis methods of clinical application of extracellular vesicles

AN Taixue, ZHENG Lei
Department of Laboratory Medicine, Nanfang Hospital of Southern Medical University, Guangzhou 510515, China

Abstract: Extracellular vesicles (EVs) are small vesicles released by cells, which contain proteins and miRNA. It is a new research field in recent years. EVs change accordingly in a variety of diseases. These vesicles can sensitively reflect the pathological changes of the body. Compared with tissue biopsy, EVs detection have the advantages of non-invasive, simple sampling and real-time monitoring. EVs are becoming new diagnostic marker. This article reviews the current status and progress of EVs in clinical application.

Keywords: extracellular vesicles; purification; diagnosis; clinical treatment; clinical application

细胞外囊泡(EVs)是指在生理和病理状态下,机体内细胞通过胞吞作用形成多泡小体后,通过细胞膜融合分泌到细胞外环境中的微小囊泡,根据囊泡直径大小,可将EVs分为三类:凋亡小体(>1000 nm)、微囊泡(100~1000 m)、外泌体(30~100 nm)[1]。EVs最初仅被视作细胞的"垃圾袋",用于清除不必要的大分子,但现在其被认为是细胞间信号的运载体,可用于细胞间通讯。EVs表面蛋白信号分子可以识别靶细胞,并通过受体配体结合或胞吞作用摄入EVs从而改变靶细胞的生理病理状态[2]。大量研究表明异常细胞分泌的EVs也出现异常;其内部的多种分子显著改变。与传统的疾病诊断标志物相比,EVs可在体液中稳定存在且半衰期长[3],加之脂质双分子层的保护作用,EVs可靶向运送RNA和蛋白质等生物信息分子至受体细胞,并且EVs广泛分布于体液中,取材方便,创伤小,因此EVs有望成为一种新型的疾病诊断标志物[4]。

收稿日期:2017-05-11
基金项目:国家自然科学基金(81371901);广州市科技计划资助项目(1563000220)
Supported by National Natural Science Foundation of China (81371901).
作者简介:安泰学,博士,电话:020-62786963,E-mail: atxhuazhong@126.com
通信作者:郑 磊,教授,博士生导师,电话:020-61642147, E-mail: nfyyzhenglei@smu.edu.cn

1 EVs标本的采集与保存

EVs存在于体内多种体液中;目前已证明存在EVs的体液包括:胸水[5]、血浆[6]、房水[7],乳汁[8],腹水[9],羊水[10],精液[11],唾液[12],鼻腔分泌物[13],脑脊液[14],支气管肺泡灌洗液[15],关节腔滑液[16],胆汁[17]以及尿液[18]。其中多种体液中的EVs在疾病情况下显著异常,有望作为诊断标志物应用于临床。各种体液标本的采集、保存是EVs临床检测的第一步,严重影响着后续EVs的纯化和检测。因此,标本采集、保存的标准化对于提高实验室结果间的可比性及加速EVs应用于临床都具有重要意义。EVs对机体状态改变十分敏感,年龄、性别、吸烟、疾病史、体质指数、服用药物、空腹、采集时间都可能对EVs产生影响[19]。因此在采集标本前应明确以上信息。目前尚无研究证明种族和人种间EVs存在差异。在各种体液中研究最多的即为血液标本,因此以下以血液标本为例简介EVs体液标本的采集、保存方法。EVs的血液标本主要分为血浆标本和血清标本。由于在凝血过程中血小板也会释放EVs[20],因此尽管尚无研究详细比较血浆、

血清间EVs的差异,大部分研究者仍采用血浆标本。国际血栓和止血学会(ISTH)科学标准化委员会(SSC)推荐采用枸橼酸钠作为EVs研究用标本的抗凝剂[21]。此外,采血中为减少血小板激活释放EVs,应采用21G针头、轻柔颠倒混匀并尽量避免溶血。采集后应尽快去除血细胞及血小板,防止细胞破裂大量释放核酸及蛋白酶类。以往研究认为miRNA能够在常温下保存较长时间,但最近的研究表明血清不同miRNA的温度敏感性不同,EVs对miRNAs虽有保护作用但仍下降到原有量的一半左右[22]。此现象尚需验证,而血液EVs标本的处理方法更需深入研究。各研究中EVs多保存在-80 ℃环境下。虽然已有研究表明多次冻融对EVs的影响较小[23-24],但尚无详细研究确定EVs的最适合的保存温度。

EVs仍属于一个较新的领域,人们对EVs的认识还远远不够,未能确定合适的体液EVs标本的采集、保存方法。因此,目前研究者仅能依据自身研究的目的方案、标本及标志物性质并参考已有的信息,确定自身合适的标本采集、保存方案。

2 EVs的分离检测手段

目前较成熟的EVs分离纯化方法包括超速离心(UC)、免疫沉淀或亲和纯化、超滤法、尺寸排阻等(表1)。实际情况下应结合实验对EVs的纯度和浓度要求进行选择。各方法都只是在某一方面对颗粒进行提纯,故纯化后的EVs纯度常较低,影响后续试验。因此发展稳定高效EVs的提取方法[25]对EVs基础研究及临床应用都具有重要意义。

EVs形态学常用检测方法有透射电子显微镜、动态光散射、纳米颗粒跟踪分析仪(Nanosight)、Western blot、流式分析技术等。其中透射电子显微镜技术可直接观察EVs形态,但对样品质量要求较高、实验前处理复杂且实验中较难辨别EVs和杂质颗粒。动态光散射和Nanosight可反映EVs的粒径分布,Nanosight可直接检测EVs的浓度,但要求样本纯度高,无法区分粒径相同的蛋白聚集体、溶液杂质及EVs。Western blot实验仅能对EVs进行定性;无法避免细胞蛋白的污染,至今仍未找到EVs特有的蛋白质标志物。流式技术能够实现,高通量、单颗粒、同时检测多个参数的分析,是目前最有潜力应用于临床的外泌体分析技术。传统的流式细胞仪检测下限为500纳米,外泌体多被当成噪音而忽略。近年来研究者不断开发新技术来满足外泌体分析要求;如Apogee公司和贝克曼公司先后推出检测下限约为100 nm的Apogee A50和Cytoflex。厦门大学颜晓梅等人也开发出超敏流式分析仪,将检测灵敏度提高到20纳米,大大提升了流式技术分析外泌体的能力。灵敏度和分辨率将不会再成为流式技术分析外泌体的瓶颈。目前,困扰流式技术分析外泌体的问题同样集中于,样品的前处理、流式方法的标准化、外泌体标志物寻找。

表1 EVs纯化方法优缺点比较

Tab.1 Advantages and disadvantages of current approaches to EV isolation

Methods	Advantages	Disadvantages
Successive differential centrifugation	High purity	Highly labour intensive, limited processing capacity, low yield and time consuming
Size-exclusion	Easy manipulation and relatively high purity	limited processing capacity and low yield
Immunomagnetic sorting,	High specificity and high purity	Low yield and there is no molecule specific to exosome
Precipitation reagents	Convenience and high yield	Susceptible to the contamination of precipitated molecules

EVs含有核酸、蛋白质以及脂质等多种生物活性物质。其中研究较多的为EVs中的蛋白质和核酸。已有较多研究采用质谱技术检测血液[26]、胸水[27]、尿液[28]、以及细胞培养上清[29]EVs中的蛋白质表达谱。但受到EVs纯化方法的限制,EVs中差异表达的蛋白质分子较难应用大规模标本进行验证。对筛选到的EVs的差异表达蛋白,大多研究采用Western blot进行验证。但Western blot方法较复杂多应用于实验室验证,较难应用于临床检测。部分研究采用ELISA的方法进行验证。如Moon等采用双抗体夹心法,CD63作为包被抗体,差异蛋白Fibronectin作为酶标抗体取得了良好的效果[30]。还有部分学者采用质谱多反应监测技术对标本中的蛋白进行绝对定量,验证差异表达的蛋白质[31-32]。在EVs核酸的研究中,研究者多采用与血清核酸类似的研究策略:采用测序或杂交芯片技术对EVs差异表达的核酸进行筛选,再用实时荧光定量PCR或数字PCR进行验证。目前,疾病中EVs异常表达的核酸分子包括DNA、miRNA及LncRNA等。现在PCR检测EVs内核酸尚未能确定稳定的内参,且引入外参的方法存在问题如:样品性状干扰外参定量。核酸校准是目前EVs核酸研究的瓶颈所在。

另外已有研究利用电化学[33]、光学[34]和微流体[35]生

物传感器检测生物样本中的EVs;生物传感器应用于EVs分离检测更有望成为一种便携装置应用于床旁检测。研究者提出了应用多种分离和检测技术对EVs进行联合分析的策略[36]。如荷兰启动一项"Cancer-ID"项目,其利用多种分离检测技术如流式细胞术、表面等离子共振技术、电子显微镜技术、拉曼光谱技术、原子力显微镜技术和RNA测序技术等分析同一个病人的EVs,致力于更为全面地分析EVs并将所得数据充分的应用于临床诊断和治疗。

3 EVs的临床应用

EVs作为"信号传导装置"穿梭于细胞间调节靶细胞的生物学功能,甚至影响细胞的微环境的形成,因此研究体液内的EVs不仅可反映机体的生理状态,也预示着疾病的发生与转归。EVs不仅可以辅助非肿瘤性疾病诊断,如EVs中的Fenituin-A可以作为急性肾损伤的潜在标志物;也可以作为肿瘤检测标志物。如Tanaka等[37]发现食管癌患者血清exosomes中miRNA-21表达水平与肿瘤分期,淋巴结累及情况和肿瘤转移相关,因此,筛选EVs内特异性的疾病标志物具有重要意义。目前,人们已经尝试将EVs中的核酸及蛋白用于疾病的诊断。研究者通过蛋白质组学找到了一些具有诊断价值的蛋白分子。Sonia A等[38]通过超高液相质谱联用发现一种细胞膜锚定蛋白GPC1在胰腺癌肿瘤细胞分泌的囊泡中特异性的高表达。其诊断胰腺癌灵敏度和特异度均为100%,明显优越于传统的CA199检测,并且血液囊泡中GPC1升高早于影像学改变。当前对EVs中的核酸研究主要集中于RNA方面,特别是miRNA。Vaksman等[39]发现卵巢癌患者exosomes中miRNA-21水平增高则患者生存率降低,提示miRNA-21可用于衡量患者病情预后和疾病转归。在尿液EVs中的miR-34a[40]和LncRNA-p21在前列腺良性增生和前列腺癌患者中有差异表达,可用于良恶性前列腺疾病的鉴别,LncRNA-p21与PSA联合检测可以显著提高前列腺癌的诊断特异性[41]。表2列出了部分将EVs作为肿瘤标志物的研究,由于篇幅限制,更多关于EVs在肿瘤中的研究进展可参阅本团队综述[42]。

4 EVs研究展望

EVs作为"液体活检"的重要组成部分在疾病的精确诊断和治疗方面具有巨大的应用前景。在疾病诊断方面,随着技术的完善,EVs成分及功能的研究越发成熟,EVs的研究呈指数增长。但目前EVs的临床应用研究较少,EVs在辅助疾病诊断和预后监测的潜力仍有待挖掘。使EVs从实验室中的差异表达物质变为临床上的成熟诊断标志物,是EVs的最重要研究方向之一。当前已有多家公司致力于将EVs应用于临床疾病诊断,如Codiak BioSciences、Exovita Biosciences 和 Exosome

表2 肿瘤病人体液EV作为肿瘤诊断标志物
Tab.2 Body fluid EVs as diagnostic markersfor tumors

Tumor type	Molecues	Body fluid	Markers	Application	Reference
Prostate cancer	Protein	Urine	β-catenin	Screening	[44]
Prostate cancer	miRNA	Plasma, serum, urine	4 miRNAs	Diagnosis, prognosis	[45]
Prostate cancer	Vesicles	Plasma	Microvesicle number	Diagnosis, prognosis	[46]
Ovarian cancer	Protein	Serum	exosomal antigen	Diagnosis	[47]
Ovarian cancer	miRNA	Serum	12 miRNAs	Screening	[48]
Lung cancer	Protein	Pleural effusion	3 proteins	Diagnosis	[49]
Lung squamous cell carcinoma	miRNA	Plasma	5 miRNAs	Monitor	[50]
Lung adenocarcinoma cancer	miRNA	Plasma	10 miRNAs	Screening	[51]
GBM	miRNA	Serum	3 miRNAs	Diagnosis	[52]
Breast Cancer	miRNA	Serum	3 miRNAs	Diagnosis	[53]
Pancreatic cancer	mRNA	Saliva	7 mRNAs	Diagnosis	[54]
Colon cancer	Protein	Ascites	Claudin-3	Diagnosis	[55]
Melanoma	Protein	Plasma	Caveolin-1	Diagnosis, prognosis	[56]
Gastric cancer	Vesicle	Plasma	EVs concentration	Diagnosis	[57]
Bladder Cancer	Protein	Serum	EPS812, mucin-4	Diagnosis	[58]
Cervical cancer	miRNA	Cervical lavage fluid	miR-21, miR-146a	Diagnosis	[59]
Acute lymphoma	miRNA	Plasma	miR-92	Diagnosis	[60]
Liver cancer	Vesicles	Serum	Microvesicle number	Diagnosis	[61]

Diagnostics等。2016年1月21日,Exosome Diagnostics推出了世界上第一个分析血液外泌体RNA的液体活检试剂盒ExoDx™ Lung。样本量超过30 000例患者的实验表明其灵敏度和特异度达到88%和100%。它可通过血浆中外泌体RNA,灵敏、准确、实时监测非小细胞肺癌患者的EML4-ALK融合,实现非小细胞肺癌患者的个体化治疗。在疾病治疗方面,利用EVs稳定存在于体液中、可以靶向识别细胞或组织、克服生物屏障等特性,研究人员正在开发EVs改造技术,使其成为更有针对性的运载工具,用于靶向运输药物或生物治疗分子[43]。目前,EVs的研究方兴未艾,有大量关键的问题需要解决,如EVs的提取、分类以及EVs在疾病中的作用。随着越来越多的学者重视EVs在胞间信息传递中的作用,EVs研究进程大幅提速。近期不断有重要成果出现,相信不久的将来会有更多的EVs诊断治疗产品进入临床。

参考文献:

[1] Baranyai T, Herczeg K, Onódi Z, et al. Isolation of exosomes from blood plasma: qualitative and quantitative comparison of ultracentrifugation and size exclusion chromatography methods[J]. PLoS One, 2015, 10(12): e0145686.

[2] Tkach M, Théry C. Communication by extracellular vesicles: where we are and where we need to go[J]. Cell, 2016, 164(6): 1226-32.

[3] Jin Y, Chen K, Wang Z, et al. DNA in serum extracellular vesicles is stable under different storage conditions[J]. BMC Cancer, 2016, 16(1): 753.

[4] 黄依瑶, 郑 磊. 重视外泌体的实验诊断价值[J]. 中华检验医学杂志, 2015, 38(11): 724-6.

[5] Lin J, Wang Y, Zou YQ, et al. Differential miRNA expression in pleural effusions derived from extracellular vesicles of patients with lung cancer, pulmonary tuberculosis, or pneumonia [J]. Tumor Biology, 2016, 37(12): 15835-45.

[6] Huang X, Yuan T, Liang M, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer[J]. Eur Urol, 2015, 67(1): 33-41.

[7] Perkumas KM, Hoffman EA, Mckay BS, et al. Myocilin-associated exosomes in human ocular samples[J]. Exp Eye Res, 2007, 84(1): 209-12.

[8] Admyre C, Johansson SM, Qazi KR, et al. Exosomes with immune modulatory features are present in human breast milk [J]. J Immunol, 2007, 179(3): 1969-78.

[9] Dai S, Wei D, Wu Z, et al. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer[J]. Mol Ther, 2008, 16(4): 782-90.

[10] Asea A, Jean-Pierre C, Kaur P, et al. Heat shock protein-containing exosomes in mid-trimester amniotic fluids[J]. J Reprod Immunol, 2008, 79(1): 12-7.

[11] Poliakov A, Spilman M, Dokland T, et al. Structural heterogeneity and protein composition of Exosome-Like vesicles (prostasomes) in human semen[J]. Prostate, 2009, 69(2): 159-67.

[12] Berckmans RJ, Sturk A, Van Tienen LM, et al. Cell-derived vesicles exposing coagulant tissue factor in saliva[J]. Blood, 2011, 117(11): 3172-80.

[13] Lässer C, O'neil SE, Ekerljung L, et al. RNA-containing exosomes in human nasal secretions[J]. Am J Rhinol Allergy, 2011, 25(2): 89-93.

[14] Street JM, Barran PE, Mackay CL, et al. Identification and proteomic profiling of exosomes in human cerebrospinal fluid[J]. J Transl Med, 2012, 10: 5.

[15] Levänen B, Bhakta NR, Torregrosa Paredes P, et al. Altered microRNA profiles in bronchoalveolar lavage fluid exosomes in asthmatic patients[J]. J Allergy Clin Immunol, 2013, 131(3): 894-903.

[16] Fourcade O, Simon MF, Viodé C, et al. Secretory phospholipase A2 generates the novel lipid mediator lysophosphatidic acid in membrane microvesicles shed from activated cells[J]. Cell, 1995, 80 (6): 919-27.

[17] Masyuk AI, Huang BQ, Ward CJ, et al. Biliary exosomes influence cholangiocyte regulatory mechanisms and proliferation through interaction with primary cilia[J]. Am J Physiol Gastrointest Liver Physiol, 2010, 299(4): G990-9.

[18] Raj DA, Fiume I, Capasso G, et al. A multiplex quantitative proteomics strategy for protein biomarker studies in urinary exosomes[J]. Kidney Int, 2012, 81(12): 1263-72.

[19] Witwer KW, Buzás EI, Bemis LT, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research[J]. J Extracell Vesicles, 2013, 2. doi: 10.3402/jev.v2i0.20360.

[20] Gemmell CH, Sefton MV, Yeo EL. Platelet-derived microparticle formation involves glycoprotein IIb-IIIa. Inhibition by RGDS and a Glanzmann's thrombasthenia defect [J]. J Biol Chem, 1993, 268 (20): 14586-9.

[21] Lacroix R, Judicone C, Mooberry M, et al. Standardization of pre-analytical variables in plasma microparticle determination: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop[J]. J Thromb Haemost, 2013 doi: 10.1111/jth.12207.

[22] Aiso T, Takigami S, Yamaki A, et al. ANNALS EXPRESS: Degradation of serum microRNAs during transient storage of serum samples at 4 degrees C [J]. Ann Clin Biochem, 2017: 1962435881.

[23] Jayachandran M, Miller VM, Heit JA, et al. Methodology for isolation, identification and characterization of microvesicles in peripheral blood[J]. J Immunol Methods, 2012, 375(1/2): 207-14.

[24] Sokolova V, Ludwig AK, Hornung S, et al. Characterisation of exosomes derived from human cells by nanoparticle tracking analysis and scanning electron microscopy [J]. Colloids Surf B Biointerfaces, 2011, 87(1): 146-50.

[25] Oksvold MP, Neurauter A, Pedersen KW. Magnetic bead-based isolation of exosomes[J]. Methods Mol Biol, 2015, 1218: 465-81.

[26] Yentrapalli R, Merl-Pham J, Azimzadeh O, et al. Quantitative changes in the protein and miRNA cargo of plasma exosome-like vesicles after exposure to ionizing radiation[J]. Int J Radiat Biol, 2017, 93(6): 569-80.

[27] Park JO, Choi DY, Choi DS, et al. Identification and characterization of proteins isolated from microvesicles derived from human lung cancer pleural effusions[J]. Proteomics, 2013, 13 (14): 2125-34.

（下转封三页）

（上接1562页）

[28] Fujita K, Kume H, Matsuzaki K, et al. Proteomic analysis of urinary extracellular vesicles from high Gleason score prostate cancer[J]. Sci Rep, 2017, 7: 42961.
[29] Sinha A, Alfaro J, Kislinger T. Characterization of protein content present in exosomes isolated from conditioned media and urine[J]. Curr Protoc Protein Sci, 2017, 87: 24.9.1-24.9.12.
[30] Moon PG, Lee JE, Cho YE, et al. Fibronectin on circulating extracellular vesicles as a liquid biopsy to detect breast cancer[J]. Oncotarget, 2016, 7(26): 40189-99.
[31] Chen CL, Lai YF, Tang P, et al. Comparative and targeted proteomic analyses of urinary microparticles from bladder cancer and hernia patients[J]. J Proteome Res, 2012, 11(12): 5611-29.
[32] Kruh-Garcia NA, Wolfe LM, Chaisson LH, et al. Detection of mycobacterium tuberculosis peptides in the exosomes of patients with active and latent M. tuberculosis infection using MRM-MS[J]. PLoS One, 2014, 9(7): e103811.
[33] Jeong S, Park J, Pathania D, et al. Integrated Magneto-Electrochemical sensor for exosome analysis[J]. ACS Nano, 2016, 10(2): 1802-9.
[34] Im H, Shao H, Park YI, et al. Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor [J]. Nat Biotechnol, 2014, 32(5): 490-5.
[35] Santana SM, Antonyak MA, Cerione RA, et al. Microfluidic isolation of cancer-cell-derived microvesicles from hetergeneous extracellular shed vesicle populations [J]. Biomed Microdevices, 2014, 16(6): 869-77.
[36] Van Der Pol E, Böing AN, Gool EL, et al. Recent developments in the nomenclature, presence, isolation, detection and clinical impact of extracellular vesicles[J]. J Thromb Haemost, 2016, 14(1): 48-56.
[37] Tanaka Y, Kamohara H, Kinoshita KA, et al. Clinical impact of serum exosomal microRNA-21 as a clinical biomarker in human esophageal squamous cell carcinoma [J]. Cancer, 2013, 119(6): 1159-67.
[38] Melo SA, Luecke LB, Kahlert C, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer[J]. Nature, 2015, 523 (7559): 177-82.
[39] Vaksman O, Tropé C, Davidson B, et al. Exosome-derived miRNAs and ovarian carcinoma progression[J]. Carcinogenesis, 2014, 35(9): 2113-20.
[40] Corcoran C, Rani S, O'driscoll L. miR-34a is an intracellular and exosomal predictive biomarker for response to docetaxel with clinical relevance to prostate cancer progression[J]. Prostate, 2014, 74(13): 1320-34.
[41] Işın M, Uysaler E, Özgür E, et al. Exosomal lncRNA-p21 levels May help to distinguish prostate cancer from benign disease [J]. Front Genet, 2015, 6: 168.
[42] An T, Qin S, Xu Y, et al. Exosomes serve as tumour markers for personalized diagnostics owing to their important role in cancer metastasis[J]. J Extracell Vesicles, 2015, 4: 27522.
[43] Vader P, Mol EA, Pasterkamp G, et al. Extracellular vesicles for drug delivery[J]. Adv Drug Deliv Rev, 2016, 106(Pt A): 148-56.
[44] Lu Q, Zhang J, Allison R, et al. Identification of extracellular delta-catenin accumulation for prostate cancer detection [J]. Prostate, 2009, 69(4): 411-8.
[45] Bryant RJ, Pawlowski T, Catto JW, et al. Changes in circulating microRNA levels associated with prostate cancer[J]. Br J Cancer, 2012, 106(4): 768-74.
[46] Tavoosidana G, Ronquist G, Darmanis S, et al. Multiple recognition assay reveals prostasomes as promising plasma biomarkers for prostate cancer[J]. Proc Natl Acad Sci USA, 2011, 108(21): 8809-14.
[47] Taylor DD, Gercel-Taylor C, Parker LP. Patient-derived tumor-reactive antibodies as diagnostic markers for ovarian cancer [J]. Gynecol Oncol, 2009, 115(1): 112-20.
[48] Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer [J]. Gynecol Oncol, 2008, 110(1): 13-21.
[49] Bard MP, Hegmans JP, Hemmes A, et al. Proteomic analysis of exosomes isolated from human malignant pleural effusions[J]. Am J Respir Cell Mol Biol, 2004, 31(1): 114-21.
[50] Aushev VN, Zborovskaya IB, Laktionov KK, et al. Comparisons of microRNA patterns in plasma before and after tumor removal reveal new biomarkers of lung squamous cell carcinoma[J]. PLoS One, 2013, 8(10): e78649.
[51] Cazzoli R, Buttitta F, Di Nicola M, et al. microRNAs derived from circulating exosomes as noninvasive biomarkers for screening and diagnosing lung cancer[J]. J Thorac Oncol, 2013, 8(9): 1156-62.
[52] Manterola L, Guruceaga E, Gállego Pérez-Larraya J, et al. A small noncoding RNA signature found in exosomes of GBM patient serum as a diagnostic tool[J]. Neuro Oncol, 2014, 16(4): 520-7.
[53] Rupp AK, Rupp C, Keller S, et al. Loss of EpCAM expression in breast cancer derived serum exosomes: role of proteolytic cleavage [J]. Gynecol Oncol, 2011, 122(2): 437-46.
[54] Lau C, Kim Y, Chia D, et al. Role of pancreatic cancer-derived exosomes in salivary biomarker development [J]. J Biol Chem, 2013, 288(37): 26888-97.
[55] Choi DS, Park JO, Jang SC, et al. Proteomic analysis of microvesicles derived from human colorectal cancer ascites[J]. Proteomics, 2011, 11(13): 2745-51.
[56] Logozzi M, De Milito A, Lugini L, et al. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients [J]. PLoS One, 2009, 4(4): e5219.
[57] Kim HK, Song KS, Park YS, et al. Elevated levels of circulating platelet microparticles, VEGF, IL-6 and RANTES in patients with gastric cancer: possible role of a metastasis predictor [J]. Eur J Cancer, 2003, 39(2): 184-91.
[58] Smalley DM, Sheman NE, Nelson K, et al. Isolation and identification of potential urinary microparticle biomarkers of bladder cancer [J]. J Proteome Res, 2008, 7(5): 2088-96.
[59] Liu J, Sun H, Wang X, et al. Increased exosomal microRNA-21 and microRNA-146a levels in the cervicovaginal lavage specimens of patients with cervical cancer[J]. Int J Mol Sci, 2014, 15(1): 758-73.
[60] Tanaka M, Oikawa K, Takanashi M, et al. Down-regulation of miR-92 in human plasma is a novel marker for acute leukemia patients [J]. PLoS One, 2009, 4(5): e5532.
[61] Wang W, Li H, Zhou Y, et al. Peripheral blood microvesicles are potential biomarkers for hepatocellular carcinoma [J]. Cancer Biomark, 2013, 13(5): 351-7.

（编辑:吴锦雅）